

CLAIMS

1. A polynucleotide comprising a sequence capable of hybridising selectively to

- 5 (a) SEQ ID NO: 1 or the complement thereof;  
(b) a sequence from the 3.6 kb plasmid of *Propionibacterium freudenreichii* CBS 101022;  
(c) a sequence from the 3.6 kb plasmid of *Propionibacterium freudenreichii* CBS 101023; or  
10 (d) a sequence that encodes a polypeptide which comprises a SEQ. ID. No. 2 or 3, an amino acid sequence substantially homologous thereto or a fragment of either sequence.

2. A polynucleotide which is an autonomously replicating plasmid that can remain extrachromosomal inside a host cell, which plasmid is derived from an endogenous *Propionibacterium* plasmid, and when comprising a heterologous gene  
15 is capable of expressing that gene inside the host cell.

3. A polynucleotide according to claim 1 which is autonomously replicating in a host cell.

4. A polynucleotide according to claim 3 in which the host cell is a *Propionibacterium*.

20 5. A polynucleotide according to claim 4 in which the *Propionibacterium* is *Propionibacterium freudenreichii*.

\* 6. A polynucleotide according to any one of the preceding claims which is capable of selectively hybridising to one or more sequence(s) in SEQ ID No:1 which is (or are) necessary for autonomous replication in a *Propionibacterium*.

25 7. A polynucleotide according to claim 1 which comprises either the 1.7 kb fragment of SEQ. ID. No. 1 delineated by restriction sites *SaII* and *AhaNI* or nucleotides 1 to 1750 of SEQ. ID. No. 1.

\* 8. A vector which comprises a polynucleotide according to any one of the preceding claims.

30 9. A vector according to claim 8 which is a plasmid.

\* 10. A vector according to claim 8 or 9 which additionally comprises a

selectable marker.

11. A vector according to any one of claims 8 to 10 which is autonomously replicating in *E. coli*.
12. A vector according to any one of claims 8 to 11 which is an expression vector.
13. A vector according to claim 12 which comprises an endogenous gene of a *Propionibacterium* or a heterologous gene operatively linked to a control sequence which is capable of providing for expression of the gene.
14. A vector according to claim 13 in which the gene is the *cobA* gene.
15. A vector according to claim 13 in which the heterologous gene encodes a polypeptide which is therapeutic in a human or animal.
16. A polypeptide which comprises the sequence SEQ ID No: 2 or 3 or a sequence substantially homologous thereto, or a fragment of either said sequence, or is encoded by a polynucleotide as defined in any of claims 1 to 7.
17. A host cell comprising a heterogeneous polynucleotide or vector according to any one of claims 1 to 15 or which can be transformed or transfected with a vector according to any one of claims 13 to 15.
18. A host cell according to claim 17 which is a bacterium.
19. A host cell according to claim 18 which is a *Propionibacterium* or *E. coli*.
20. A process for producing a host cell according to any one of claims 17 to 19 comprising transforming or transfecting a host cell with a polynucleotide or vector according to any one of claims 1 to 15.
21. A process for the preparation of a polypeptide, or other compound, the process comprising cultivating or fermenting a host cell as defined in any one of claims 17 to 19 under conditions that allow expression or production of the polypeptide or compound.
22. A process according to claim 21 which is a fermentation process wherein the host cell is cultured in aerobic or anaerobic conditions.
23. A process according to claim 21 or 22 in which the expressed polypeptide or produced compound is recovered from the host cell.
24. A process according to claim 23 wherein the polypeptide is a protease,

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amylase, lipase or peptidase or the compound is vitamin B<sub>12</sub>.

\* 25. A process according to any one of claims 21 to 24 where the polypeptide is secreted from the host cell.

26. A process according to claim 25 in which the polypeptide is expressed on the surface of the host cell and/or the polypeptide is an antigen or immunogen.

\* 27. A polypeptide or compound prepared by a process according to any one of claims 20 to 26.

\* 28. A process for the production of vitamin B<sub>12</sub> (cobalamin), the process comprising culturing a host cell according to any one of claims 17 to 19 under conditions in which the vitamin is produced and, if necessary, isolating the vitamin.

29. Vitamin B<sub>12</sub> produced by a process according to claim 28.

30. A polypeptide according to claim 27 for use in a method of treating the human or animal body by therapy.

\* 31. A host cell according to any one of claims 17 to 19 for use in a method of treating the human or animal body by therapy or for use in an animal feed.

\* 32. Use of a host cell according to any one of claims 17 to 19 or a polypeptide or compound according to claim 27 to either make cheese or for use in cheesemaking.

\* 33. Use of a host cell according to any one of the claims 17 to 19 or a polypeptide or compound according to claim 27, in the manufacture of a foodstuff or in an animal feed.

\* 34. A foodstuff comprising a polypeptide or compound according to claim 27 or a host cell according to any of claims 17 to 19.

35. A foodstuff according to claim 34 for consumption by humans (e.g. a cheese, sausage) or by an animal.

\* 36. A process for manufacturing cheese or other fermented dairy product the process comprising using a host cell according to any of claims 17 to 19.

37. A process according to claim 36 wherein the host cell is used instead of or in addition to lactic acid bacteria.

\* 38. A process according to claim 36 or 37 wherein the host cell is a *Propionibacterium* cell.

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